Cell, Vol. 61, 61–72, April 6, 1990, Copyright © 1990 by Cell Press

Drosophila Ribosomal RNA Genes Function as an X-Y Pairing Site during Male Meiosis

Bruce D. McKee* **and Gary H. Karpen†**
* Department of Biology
University of Wisconsin
Eau Claire, Wisconsin 54701
† Carnegie Institute of Washington
115 West University Parkway
Baltimore, Maryland 21210

Summary

In Drosophila melanogaster males, the sex chromosomes pair during meiosis in the centric X heterochromatin and at the base of the short arm of the Y (YS), in the vicinity of the nucleolus organizers. X chromosomes deficient for the pairing region segregate randomly from the Y. In this report we show that a single ribosomal RNA (rRNA) gene stimulates X-Y pairing and disjunction when inserted onto a heterochromatically deficient X chromosome by P element–mediated transformation. We also show that insert-containing X chromosomes pair at the site of insertion, that autosomal rDNA inserts do not affect X-Y pairing or disjunction, and that the strength of an X pairing site is proportional to the dose of ectopic rRNA genes. These results demonstrate that rRNA genes can promote X-Y pairing and disjunction and imply that the nucleolus organizers function as X-Y pairing sites in wild-type Drosophila males.

Introduction

The regular disjunction of homologous chromosomes during meiosis is responsible for Mendelian segregation and for the maintenance of euploidy in sexually reproducing species. Many of the cellular structures responsible for chromosome segregation, such as microtubule arrays (Mitchison, 1988), centrioles (McIntosh, 1983), and kinetochores (Rieder, 1982), are beginning to be understood in terms of their molecular structures and mechanisms of function. One of the least understood processes in meiosis is the mutual recognition and pairing of homologs that occurs during early prophase. Pairing is essential for subsequent meiotic events, including segregation; a chromosome that fails to pair typically disjoins randomly from its homolog and may be lost during the meiotic or subsequent mitotic divisions. Cytological and genetical analyses have provided some insights into the process of pairing, but the underlying molecular mechanisms are entirely mysterious.

An important step in understanding how homologs pair would be to discover where they pair. Do all sequences participate equally in homolog recognition or are there specific chromosomal sites at which pairing occurs? Ultrastructural studies suggest that pairing typically initiates at one or a few sites per chromosome arm and then spreads in a zipper-like fashion (Giroux, 1988). It is not clear, however, whether the initiation sites are specific or randomly chosen. The numerous reports of nonlinear pairing configurations, such as translocation crosses and inversion loops, in pachytene chromosomes of individuals heterozygous for rearrangements (reviewed in von Wettstein et al., 1984) suggest that, for most chromosomes, there must be a substantial number of pairing sites per chromosome. In an extensive study of recombination between pairs of overlapping inversions in Drosophila females, recombination was observed in all tested intervals, suggesting that sites for local initiation of pairing are widespread (Craymer, 1981).

If specific pairing sites exist, it should be possible to identify them by mutations or rearrangements that disrupt chromosome pairing in *cis*. However, genetic analysis of pairing has been hampered by the complex relationships between pairing and disjunction in most organisms. These complications include the requirement for chiasmata (the products of exchange) to stabilize bivalents (Hawley, 1988) and the existence of a "back-up" distributive disjunctional system specific for nonexchange chromosomes (Grell, 1976). In Drosophila males, the analysis of pairing is facilitated by a relatively simple relationship between pairing and disjunction. Exchange and its associated structures—synaptonemal complexes and chiasmata—are absent (Morgan, 1912; Meyer, 1960), and there is no distributive pairing (Holm, 1976). Because of this simplicity, it has been possible, by cytogenetic mapping, to partially localize sites involved in pairing. For chromosome 2, which is a large metacentric autosome with heterochromatin surrounding the centromere on both sides, pairing appears to be restricted to the euchromatic regions. Free duplications containing only second chromosome heterochromatin do not pair either with each other or with complete second chromosomes (Yamamoto, 1979), and iso–second chromosomes (attached-2L and attached-2R) with overlap only in the heterochromatin disjoin randomly from each other (Hilliker et al., 1982). Conversely, the pairing sites of the nearly telocentric X chromosome are restricted to the centric heterochromatin of the large left arm. Deletions for XL heterochromatin disrupt X-Y pairing and lead to frequent X-Y nondisjunction (Muller and Painter, 1932; Gershenson, 1933; Cooper, 1964).

More detailed mapping of the sex chromosome pairing sites has shown them to be very closely linked to the nucleolus organizers, which are located in the central half of the X heterochromatin and at the base of the short arm of the Y (Ritossa, 1976). All free X duplications that fail to disjoin regularly from an attached XY are NO^- (Lindsley and Sandler, 1958). All X heterochromatic deficiencies that disrupt X-Y pairing and disjunction to one degree or another are completely *bobbed-lethal* (recessive lethal due to rDNA insufficiency), and most *bobbed-lethal* deficiencies are also pairing-negative (McKee and Lindsley, 1987). The few apparent exceptions probably reflect different thresholds for pairing and viability rather than separa-

bility of the responsible loci. One *bobbed-lethal*, pairing-positive deficiency ($In(1)w^{m4L}w^{m51bR}$) retains 6–8 rRNA genes, as estimated by in situ hybridization to mitotic chromosomes (Appels and Hilliker, 1982). The others have not been tested molecularly. In addition to being closely linked to the rDNA, the pairing site is also functionally repetitive. Deletions for either the distal or proximal half of the heterochromatin (and of the rDNA) do not disrupt pairing (Appels and Hilliker, 1982; Lindsley et al., 1982; McKee and Lindsley, 1987). An alternative interpretation of the mapping data, first suggested by Cooper (1964), is that pairing sites are located on both sides of the nucleolus organizer. This proposal was based on Cooper's light microscope observation that X chromosomes pair either just distal or just proximal to the secondary constriction (NO). It is very difficult to determine, by cytological or cytogenetic analyses, whether two heterochromatic functions are controlled by a single locus or different, closely linked loci. The observation that two X heterochromatic deletions presumed to differ only in the amount of residual rDNA (based on the way in which they were generated) differ from each other in pairing ability (Appels and Hilliker, 1982) favors the hypothesis that the pairing site is the nucleolus organizer. However, it is difficult to be certain about the heterochromatic makeup of these deletions. A molecular test of the pairing ability of rDNA is necessary to decide if rDNA is responsible for X-Y pairing.

In addition to the NO and the X pairing site, the X heterochromatin also contains a function essential for normal sperm development. Males deficient for X heterochromatin experience spermatid mortality (Peacock et al., 1975), which results in progeny ratio distortion (Gershenson, 1933; Sandler and Braver, 1954; Peacock, 1965). There is a strong correlation between the amount of nondisjunction and the severity of distortion exhibited by different X heterochromatic deficiencies (McKee and Lindsley, 1987). A similar correlation is seen among males with the same deficiency but differing either in background genotype or in rearing temperature (Zimmering, 1963; Peacock and Miklos, 1973; Peacock et al., 1975). These correlations, and the apparent inseparability of the X heterochromatic sites responsible for distortion from the pairing site (McKee and Lindsley, 1987), have led to the proposal that disruption of X-Y pairing is directly responsible for distortion (Baker and Carpenter, 1972; Peacock and Miklos, 1973; McKee and Lindsley, 1987).

Both of these hypotheses, that the nucleolus organizer is the X-Y pairing site and that X-Y pairing is required for normal sperm development, can be tested directly using P element transformation of cloned ribosomal RNA (rRNA) genes. The prediction is that transformation of a threshold number of copies of rRNA genes onto a $rDNA^{-}$, pairing deficient X chromosome, should restore pairing ability and rescue both the nondisjunction and progeny ratio distortion phenotypes. Because of the size of rRNA genes in Drosophila (approximately 12 kb), such an experiment is feasible only if the copy number threshold for at least partial pairing is rather low. As pointed out above, X heterochromatic deficiencies with only a few (6–8) residual copies of the rRNA genes can pair normally. This suggests that the threshold may be within the range of molecular experimentation.

A previous report (Karpen et al., 1988) described the construction and germline transformation of a P transposon called p(rib,ry)7, which contains a single, uninterrupted rRNA gene. One X-linked and three autosomal insertions of p(rib,ry)7 (referred to as [rib7]) were shown to be transcribed and to induce formation of a mininucleolus at the site of insertion in salivary gland polytene chromosomes. In this report we show that [rib7] stimulates X-Y pairing and disjunction and reduces progeny ratio distortion when located on a heterochromatically deficient X but not when located autosomally, and that the levels of both X-Y nondisjunction and progeny ratio distortion are inversely proportional to the X-linked copy number of [rib7]. These results imply that rRNA genes function as X-Y pairing sites and that normal sperm development requires X-Y pairing.

Results

A Single Ectopic rRNA Gene Partially Rescues the Meiotic Defects of a Heterochromatically Deficient X Chromosome

The ability of an X-linked rRNA gene to promote X-Y pairing was tested by recombining [rib7](1A1–4) (see Figure 1 for structure) onto *Df(1)X-1*, a large heterochromatic deficiency that disjoins at random from the Y (McKee and Lindsley, 1987). [rib7](1A1–4) is located very near the tip of the X in bands 1A1–4 of the polytene chromosome map, based on in situ hybridization to salivary gland chromosomes (see Figure 5). This insert was shown in Karpen et al. (1988) (where it is referred to as [rib7](1A)) to be autonomously functional with respect to transcriptional ability and nucleolus formation. The recombinant, [rib7](1A1–4), *Df(1)X-1*, and a nonrecombinant *Df(1)X-1* chromosome were isolated from the same female to minimize genetic background differences.

Cytologically, pairing activity of [rib7](1A1–4) should be reflected both in an enhanced frequency of X-Y bivalents and in an unusual pairing configuration—with the X paired at its tip instead of near its base. To test these predictions, orcein-stained prophase I and metaphase I chromosomes were prepared from males carrying the sibling X chromosomes described above or a wild-type X. The wild-type bivalents showed the usual pattern with the X paired in the heterochromatin (Figure 2A). Bivalents were rare (0%–5%) in *Df(1)X-1* males as reported previously (McKee and Lindsley, 1987); the typical univalent pattern is shown in Figure 2B. Only an approximate estimate of bivalent frequency could be obtained from [rib7](1A1–4), *Df(1)X-1* males because the frequency varied from stage to stage, declining as anaphase I approached, and also because premature X-Y disjunction (a common occurrence in these males) was often indistinguishable from cases in which the X and Y had never paired. However, the frequency of X-Y bivalents was significantly higher during prophase in [rib7](1A1–4), *Df(1)X-1* males (20%–40%) than in *Df(1)X-1* males. Striking confirmation of the terminal pairing prediction was obtained for [rib7](1A1–4), *Df(1)X-1*

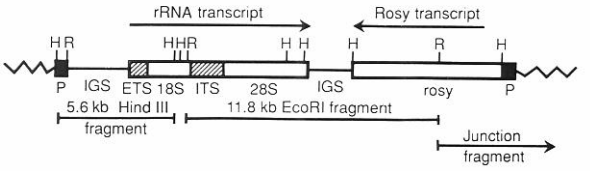

Figure 1. Structure of [rib7]

Partial restriction map of [rib7] insertion (redrawn from Karpen, 1987); H = HindIII; R = EcoRI; P = sequences derived from the ends of a P element; IGS = intergenic spacer (also known as nontranscribed spacer); ETS = external transcribed spacer; 18S = 18S rRNA sequence; ITS = internal transcribed spacer; 28S = 28S rRNA sequence. The jagged lines represent flanking chromosomal segments.

males (Figures 2C–2F). In all cases in which the X-Y bivalent was sufficiently stretched for observation, the tip of one X chromatid was associated with the Y. We have never observed pairing of the X tip in the absence of an rDNA insertion. The reason for single chromatid pairing is unknown. Figure 2 also shows that the paired X chromatid frequently appears elongated. It is not clear whether this reflects stretching of the chromatid or the presence of the "thread-like" (Cooper, 1964) or "amorphous fibrillar" (Ault et al., 1982) material described previously as connecting wild-type X and Y chromosomes.

Increased X-Y pairing should result in elevated levels of X-Y disjunction. The disjunction frequency was measured cytologically by scoring orcein-stained anaphase I, metaphase II, and anaphase II figures from testis squashes for the presence or absence of the X and Y chromosomes, and genetically, by crossing the males to appropriately marked females and measuring the recovery of the four sperm classes (X, Y, XY, and O) in the progeny. The cytological method is more direct, but the genetic method allows sampling of many more meioses. For both assays the disjunction frequency is defined as the fraction of secondary spermatocytes with an X or Y but not both $= (X + Y)/(X + Y + XY + O)$. Since unpaired chromosomes disjoin at random in these males (McKee and Lindsley, 1987), the disjunction frequency is 50% in the absence of pairing compared to the nearly 100% disjunction observed in wild-type males. To estimate the disjunction frequency from progeny test data, it is necessary to compensate for the skewed progeny ratios caused by differential spermatid mortality. A reliable method of doing this is described in Experimental Procedures. In both assays the disjunction frequency proved to be substantially higher in the [rib7](1A1–4), *Df(1)X-1* males than in the *Df(1)X-1* controls: 64% versus 51% in the cytological assay and 68% versus 55% in the progeny test (Table 1). Both differences are highly significant, based on the z test. Substituting B^SY for B^SYy^+ gave substantially the same result (Table 1). Other Ys could not be tested because the B^S duplication is needed to complement the proximal euchromatic deletion in *Df(1)X-1*.

[rib7](1A1–4) also ameliorates progeny ratio distortion. Sex chromosome recovery percentages improved from 7.5% to 44% for the Y and from 30% to 67% for the X (Table 1). Similar results were obtained with B^SY. These results confirm previous evidence for a causal link between X-Y pairing and normal sperm development (Baker and Carpenter, 1972; Peacock and Miklos, 1973; McKee and Lindsley, 1987). Any factor that alters the probability of X-Y pairing (temperature, background genotype, size of X heterochromatic deletion, or, in this case, the presence of a rRNA gene) causes correlated changes in X-Y disjunction and spermatid viability.

Removal of [rib7](1A1–4) Restores Pairing Defects of *Df(1)X-1*

To be sure that the pairing stimulation is due to the inserted element itself, [rib7](1A1–4) was removed from [rib7](1A1–4), *Df(1)X-1* by three different methods: replacement of the tip by recombination with a wild-type X, dele-

Table 1. X-Y Disjunction with and without [rib7](1A1-4)

A. Testes Squash Data

Paternal X Chromosome	Ectopic rDNA Dose	Anaphase I		Meiosis II			
		X-Y (a)	XY-O (b)	O (c)	X or Y (d)	XY (e)	P ± CI
Df(1)X-1	0	23	19	53	108	57	.51 ± .06
[rib7](1A1–4), Df(1)X-1	1	18	9	52	166	46	.64 ± .05

Males carried B^SYy^+ in addition to the indicated X. Details of the testis squash method are in the Experimental Procedures. P (the disjunction frequency) was calculated from the formula $P = (2a + d)/(2a + 2b + c + d + e)$.

B. Progeny Count Data

Paternal Genotype		Sperm Class Totals				Parameters		
[rib7]	Y	X	Y	XY	O	P ± CI	R_X ± CI	R_Y ± CI
Absent	B^SYy^+	871	217	54	2402	.55 ± .02	.30 ± .02	.075 ± .009
Present	B^SYy^+	2572	1693	523	1771	.68 ± .01	.67 ± .03	.44 ± .02
Absent	B^SY	144	88	6	785	.62 ± .03	.11 ± .06	.068 ± .004
Present	B^SY	1025	707	110	547	.78 ± .02	.54 ± .02	.37 ± .02

Df(1)X-1 males with or without [rib7](1A1–4) and carrying either B^SYy^+ or B^SY were crossed singly to ry^2 females. P is the frequency of X-Y disjunction. R_X and R_Y are the recovery frequencies of the X and Y chromosomes, respectively. See Experimental Procedures for methods of calculating parameters. CI is the 95% confidence interval.

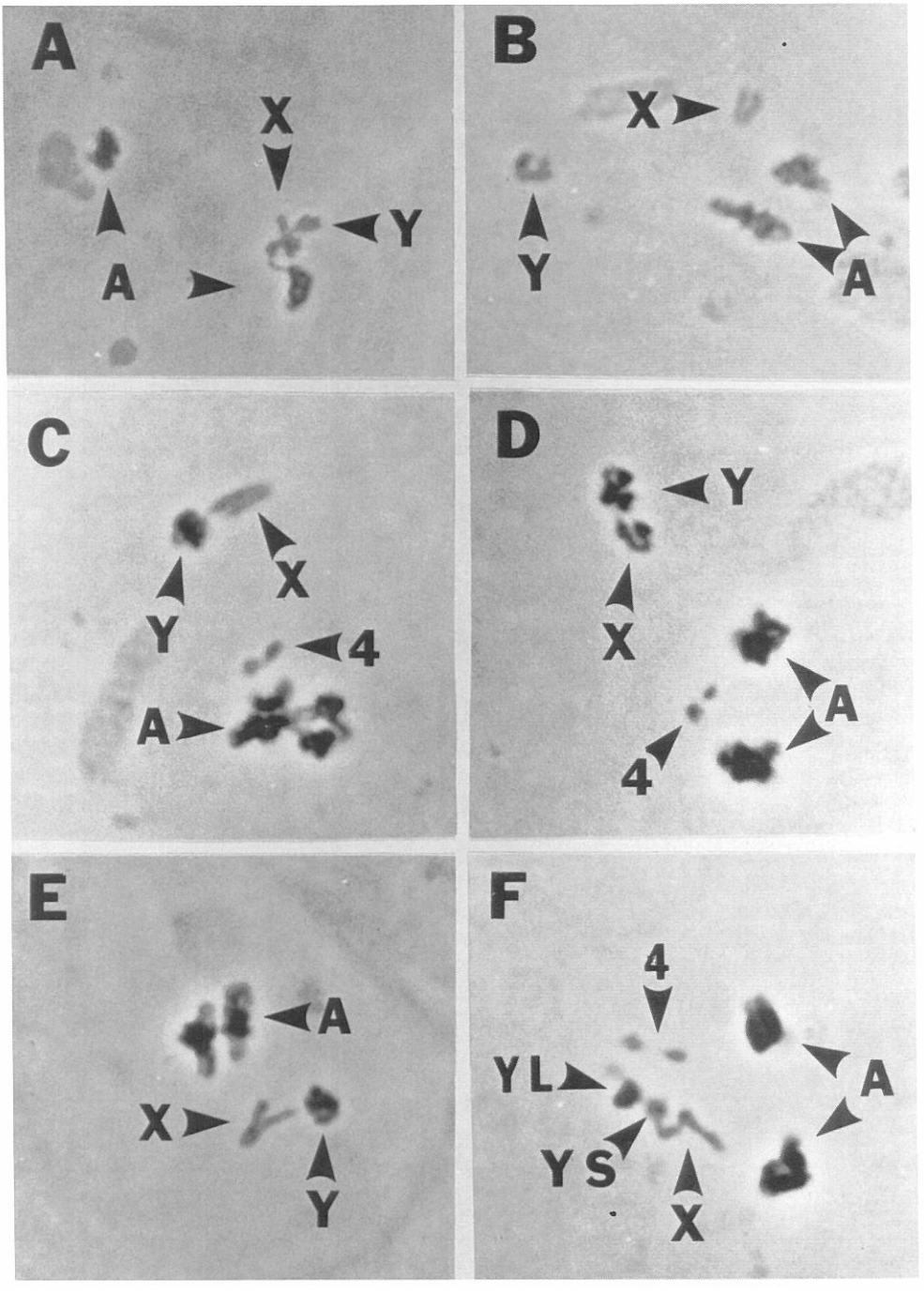

Figure 2. Terminal Pairing of [rib7](1A1–4)

Orcein-stained late prophase I and metaphase I meiotic figures from males carrying various X chromosomes and $B^{S}Yy^{+}$. Bivalents involving the major autosomes (marked A) appear the same in all males.

(A) Wild-type X. The X and Y are both paired centrally, lying over each other in this case. Unpaired tips are visible.

(B) *Df(1)X-1*: the X and Y are unpaired.

(C–F) [rib7](1A1–4), *Df(1)X-1*: four examples of X-Y bivalents with the X paired terminally. Both X chromatids are generally visible along their euchromatic extent, though the two heterochromatic Y chromatids generally appear as one. Usually only one X chromatid appears paired with the Y (D may represent an exception). Magnification: 1500×.

tion of [rib7] by γ-ray mutagenesis, and P element destabilization of [rib7] (see Experimental Procedures); the resulting $[rib7]^{-}$, *Df(1)X-1* chromosomes were tested for ability to disjoin from the Y. In each procedure the $rosy^{+}$ marker in [rib7] was used to screen for loss of the element. Each putative [rib7] deficiency was then tested by genomic Southern blotting for absence of element-specific DNA sequences (Figure 3A).

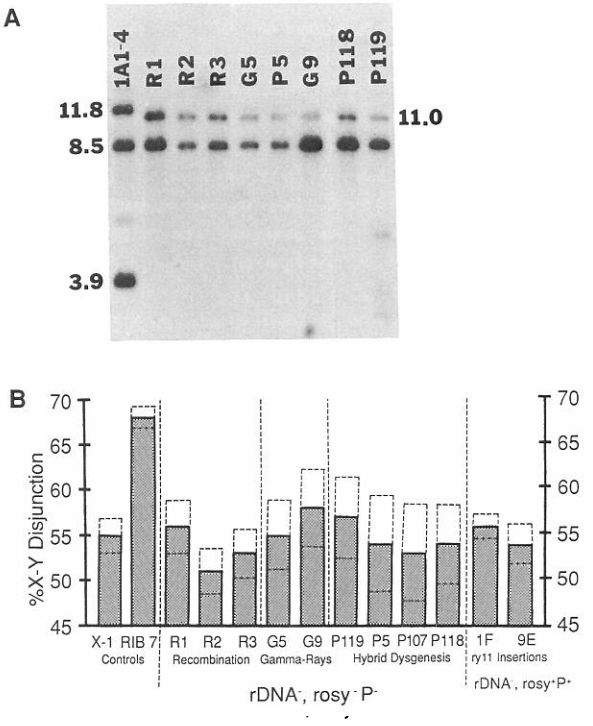

Figure 3. Reversion Tests: X-Y Disjunction in Males Carrying Insert-Deleted Derivatives of [rib7](1A1–4), *Df(1)X-1* or rDNA$^-$, *ry*$^+$ Transposons

R1, R2, and R3 are *Df(1)X-1* recombinants from [rib7](1A1–4), *Df(1)X-1/+* females. G5 and G9 are γ-ray–induced deletions of [rib7](1A1–4), *Df(1)X-1*; P119, P5, P107, and P118 are P element transposase–induced deletions of [rib7](1A1–4), *Df(1)X-1*. 1F and 9E are X chromosomal insertions of the ry11 transposon.

(A) Autoradiogram of Southern blot containing EcoRI-digested DNA from revertant lines probed with labeled Car-20. Each lane contains DNA from 5–6 adult flies. The bands characteristic of [rib7](1A1–4) (11.8 and 3.9 kb) are absent from all revertant lines; also absent is the 5.6 kb internal HindIII fragment (data not shown). See Figure 1 for restriction map. The 8.5 and 11.0 kb bands are from the *rosy* locus at 87D.

(B) The frequency of X-Y disjunction for each of the revertant lines measured by progeny testing. Dotted lines demarcate 95% confidence intervals. All males carried B^SYy^+. The controls are *Df(1)X-1* and [rib7](1A1–4), *Df(1)X-1*.

Disjunction of revertant *Df(1)X-1* chromosomes from B^SYy^+ was measured by progeny testing. The results were clear; with the exception of line R2, which showed a slightly but significantly lower disjunction percentage than the control (51% versus 55%), all [rib7]$^-$ revertants disjoined from the Y at frequencies indistinguishable from the *Df(1)X-1* control, showing that removal of [rib7] from the [rib7](1A1–4), *Df(1)X-1* chromosome eliminates its ability to pair with and disjoin from the Y (Figure 3B). This rules out the possibility that the pairing stimulation of [rib7](1A1–4) is due to a linked modifier.

Removal of [rib7] also resulted in reversion of the sperm recovery ratio phenotype. The recovery percentages of the X and Y chromosomes were indistinguishable from control *Df(1)X-1* levels in each of the rDNA$^-$ derivatives of [rib7](1A1–4) (data not shown) confirming the causal relationship between X-Y pairing and spermatid viability.

To test the formal possibility that the pairing ability of [rib7] is due to the *rosy* or P sequences rather than the rDNA, the effects of two X-linked insertions of the ry11 transposon (Spradling and Rubin, 1983) on the disjunctional ability of *Df(1)X-1* were determined. ry11 is essentially identical to p(rib,ry)7 except that it lacks the rDNA. As expected, neither of the two ry11 inserts (at 1F and 9E) stimulated X-Y disjunction (Figure 3B) or improved spermatid recovery values (data not shown). Thus, the ability of [rib7](1A1–4) to stimulate X-Y pairing is attributable to its rRNA gene.

Autosomal [rib7] Insertions Do Not Stimulate X-Y Pairing and Disjunction

If [rib7] functions as a pairing site, then it should stimulate X-Y disjunction only when located on the X. To determine the importance of genomic position, [rib7] insertions at three autosomal sites (23E on 2L, 68BC on 3L, and 94B on 3R) and three X-linked sites (1A1–4, 1A5–8, and 2EF) were compared for ability to stimulate disjunction of *Df(1)X-1* from the Y. The insertions at 1A1–4, 23E, 68BC, and 94B were described previously (Karpen et al., 1988). Those at 1A5–8 and 2EF were obtained by P element remobilization of [rib7](1A1–4) (see Experimental Procedures). The 1A5–8 insertion was accompanied by complete (and nonlethal) excision of [rib7](1A1–4). The 2EF insertion was recovered on a chromosome that retained the original 1A1–4 insertion intact. Subsequently, [rib7](2EF) was separated from [rib7](1A1–4) by recombination. Each insertion line was tested for presence of DNA fragments characteristic of [rib7] by genomic Southern blot hybridization, using probes homologous to the P and *rosy* parts of the insert. The results (Figure 4A) indicate that each single insert line contains a full-length [rib7] transposon.

For each line, the X-Y disjunction frequency in *Df(1)X-1*/B^SYy^+ males with and without the [rib7] insertion was measured by progeny testing. For the autosomal insertions, [rib7] and non-[rib7] males were siblings from a [rib7] heterozygous father. For the X insertions, the [rib7] and non-[rib7] males were taken from sibling lines derived from [rib7]/*Df(1)X-1* females. All three X-linked insertions stimulated X-Y disjunction relative to the *Df(1)X-1* control and all to the same degree (Figure 4B). Conversely, the three autosomal insertions had no effect on the frequency of X-Y disjunction when compared to sibling *Df(1)X-1* males lacking the [rib7] insertion. Thus, rDNA stimulates X-Y pairing only when it is located on the pairing-deficient X.

The ability to rescue progeny ratio distortion is also limited to X-linked [rib7] insertions. Only the three X-linked insertions improved Y chromosome recovery (Figure 4C); the figures for X chromosome recovery (not shown) follow the same pattern.

Autosomes Containing an rRNA Gene Do Not Disjoin from the Y

The ability of an autosome containing a [rib7] insertion to disjoin from the Y in the absence of competition from the X was measured for [rib7](23E) and [rib7](94B). Each [rib7] was made heterozygous with a dominantly marked homolog—SM1, *Cy* for [rib7](23E) and TM3, *Sb* for [rib7](94B)—in a *Df(1)X-1*/B^SYy^+ background. For convenience,

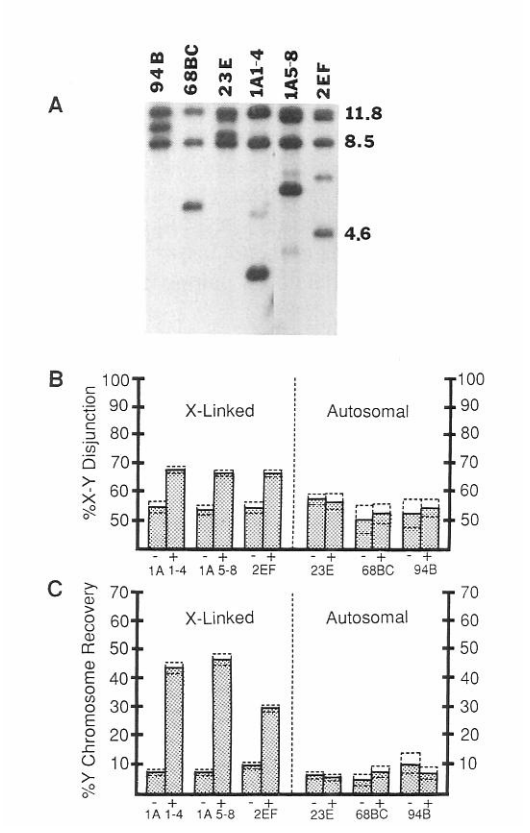

Figure 4. Effect of Autosomal versus Sex-Linked Location of [rib7] on X-Y Disjunction and Y Chromosome Recovery

(A) Autoradiogram of Southern blot containing EcoRI-digested DNA from three autosomal (94B, 68BC, and 23E) and three X-linked (1A1–4, 1A5–8, and 2EF) [rib7] lines probed with Car-20 DNA. Each lane contains DNA from 5–6 homozygous adult females. The 11.8 kb fragment is internal to [rib7] (see Figure 1) and so is present in all [rib7] lines; the 5.6 kb internal HindIII fragment is also present in all lines (data not shown). The variable band is the junction fragment from the *rosy* end of [rib7]. The 4.6 and 8.5 kb bands are from the *rosy* locus at 87D.
(B and C) The X-Y disjunction percentage measured by progeny testing (B) and the Y chromosome recovery percentage for each of the [rib7] lines and sibling non-[rib7] controls (C). All males carried *Df(1)X-1* and $B^{S}Yy^{+}$. Dotted lines bracket 95% confidence intervals.

the dominant marker on the homolog will be referred to generically as M. Sibling control males lacked the [rib7] insertion and carried a wild-type second or third chromosome instead. Disjunction of the Y from the [rib7]-containing autosome would lead to an excess of B^{S}; M and B^{+}; M^{+} offspring relative to the B^{S}; M^{+} and B^{+}; M classes (pseudolinkage). For each insert, the percentage (B^{S}; M + B^{+}; M^{+}) in the [rib7] cross was divided by the same value in the control cross (Table 2). This ratio, called NHD, is a measure of nonhomologous disjunction. It would equal 1 if there were no nonhomologous disjunction; values greater than 1 would signify pairing and disjunction of [rib7] from the Y. The observed values were 1.01 for [rib7](23E) and 0.91 for [rib7](94B). Neither is significantly different from 1. Thus, a single copy of the rDNA has no detectable pairing activity when located autosomally. This result could indicate either that rDNA can function as a pairing site only when located on the X or Y or that a single rRNA gene is shielded from exposure to the Y when trapped in an autosomal bivalent that is paired because of the activity of other, presumably stronger, pairing sites. If the latter interpretation is correct, additional copies of rDNA might overcome the shielding effect.

Increased Dosage of Ectopic rRNA Genes on *Df(1)X-1* Further Stimulates X-Y Pairing and Disjunction

If the rDNA is the sex chromosome pairing site, why is there residual nondisjunction in males carrying a single X-linked copy of [rib7]? Dosage insufficiency is a plausible explanation in light of the fact that a normal Drosophila nucleolus organizer consists of some 150–250 copies of the rDNA (Long and Dawid, 1980), while [rib7] has but 1 copy. A test of this hypothesis was made possible by the recovery of two X chromosomes containing duplications of [rib7] following P element destabilization of [rib7](1A1–4). The duplication-bearing lines were detected by the appearance of new as well as old junction fragments on genomic Southern blots (see Figure 6A) and confirmed by in situ hybridization to polytene chromosomes (Figure 5). As mentioned above, one line proved to contain both the original insertion at 1A1–4 and a second full-length insertion at 2EF. The other line, [rib7](1A1–4) × 2, has two insertions in 1A that are too close to resolve by in situ hybridization or separate by recombination. Grain counts standardized against the ry^{506} allele located at 87D show that hybridization of a *rosy*-containing probe to ectopic sequences at 1A is approximately twice as intense in this line as in the original [rib7](1A1–4) line (Figure 5). Genomic Southern blotting reveals additional junction fragments and an approximate doubling of the hybridization intensity

Table 2. Nonhomologous Disjunction (NHD) in *Df(1)X-1* Males with an Autosomal [rib7]

Paternal Autosomal Genotype	*NHD Classes*		*non-NHD Classes*		% NHD	NHD Ratio
	B^{S}; *Cy* or *Sb*	+; +	B^{S}; +	+; *Cy* or *Sb*		
[rib7](23E)/*Cy*	5	102	7	64	60	1.01
+/*Cy*	114	1837	157	1189	59	
[rib7](94B)/*Sb*	5	130	13	98	55	.91
+/*Sb*	7	100	4	62	60	

Df(1)X-1/$B^{S}Yy^{+}$; SM1, *Cy* or TM3, *Sb* males with or without the autosomal [rib7] were generated as siblings and crossed singly to *rosy* females. The NHD ratio is percent of NHD for the [rib7] males divided by percent of NHD for the control males.

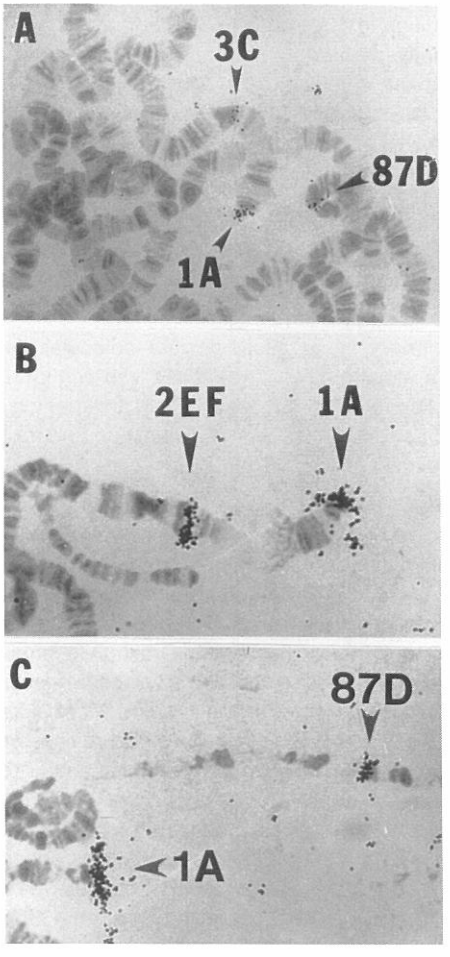

Figure 5. In Situ Hybridization of ^{3}H-Labeled *rosy* DNA to Polytene Chromosomes from Females Carrying [rib7] Insertions

[rib7](1A1–4) (A), [rib7(1A1–4) + (2EF) (B), and [rib7](1A1–4) × 2 (C). Car-20 contains sequences from the *rosy* and *white* loci in addition to the ends of a P element. The silver grains at 1A and 2EF represent hybridization of *rosy* and P sequences in Car-20 to the inserted [rib7] sequences. The silver grains at 87D and 3C represent hybridization to the *rosy* and *white* loci, respectively. To estimate the [rib7] copy number at 1A in [rib7](1A1–4) × 2 (C), the 1A:87D hybridization ratio was estimated by counting silver grains. Since this line is homozygous for the ry^{506} allele, which contains a 2.9 kb deletion in the region of homology with Car-20, the expected 1A:87D ratio is 0.649:0.351 if 1 copy is present at 1A ((7.2 kb *rosy* + 0.75 kb P):(4.3 kb *rosy*)) or 0.787:0.213 for 2 copies of the [rib7] insert. The observed numbers for the [rib7](1A1–4) × 2 line (C) were 835 grains at 1A and 248 at 87D (21 nuclei), which is consistent with the 2 copy expected ratio (χ^2 = 1.59, 1 degree of freedom). Similar calculations using the 1A:87D ratio (453:322, 28 nuclei) of the original [rib7](1A1–4) line (A) lead to an estimate of 1 copy of [rib7], as expected. Magnification: 562.5×.

to internal fragments. No new internal fragments are seen, indicating that [rib7](1A1–4) × 2 contains two full-length, unrearranged [rib7] insertions.

The effect of X-linked [rib7] copy number (from 0–2) on *Df(1)X-1*–Y disjunction was determined by both the testis squash and progeny count methods (Figure 6B). The data show a clear relationship between rDNA dose and X-Y disjunction, with the disjunction percentage increasing from

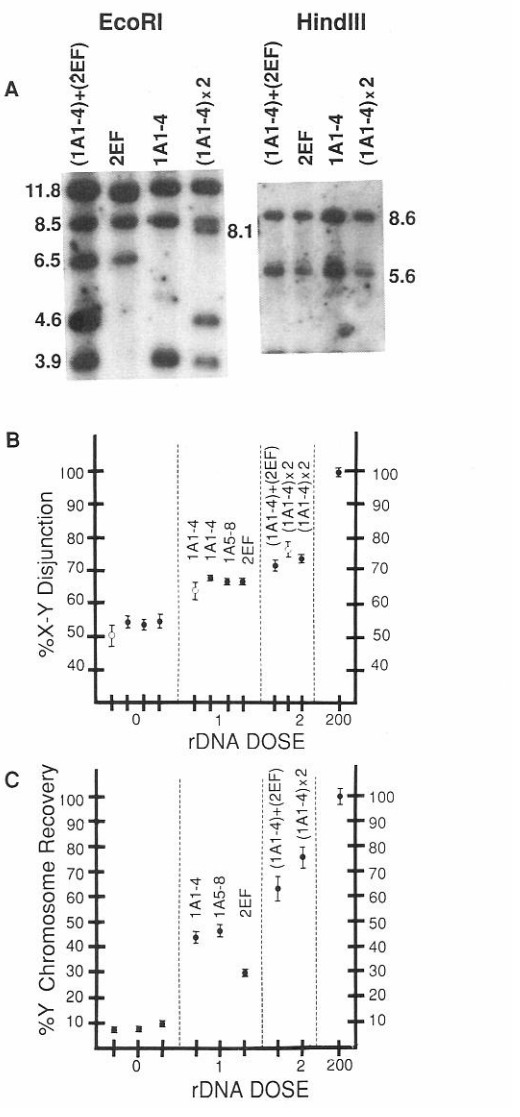

Figure 6. Effects of Ectopic rDNA Dose on X-Y Disjunction and Y Chromosome Recovery

(A) Autoradiogram of Southern blot containing DNA from homozygous lines containing 1 (1A1–4 and 2EF) and 2 ((1A1–4) + (2EF) and (1A1–4) × 2) X-linked copies of [rib7]. EcoRI digests were probed with labeled Car-20 DNA, which hybridizes predominantly to bands with *rosy* homology. HindIII digests were probed with labeled Car-2, which lacks *rosy* and hybridizes primarily to bands homologous to the P element ends. See Figure 1 for map. Each lane contains DNA from 5–6 adult females. The EcoRI band at 11.8 kb and the HindIII band at 5.6 kb are internal to [rib7]; their intensity is proportional to the dose of [rib7]. The lack of additional internal bands in the putative 2 dose lines implies that no rearrangements have occurred. The 3.9, 6.5, and 8.1 kb bands are junction fragments from the *rosy* (proximal) end of the element (see Figure 1); the number of such fragments (one in 1A1–4 and 2EF and two in (1A1–4) × 2 and (1A1–4) + (2EF)) reflects the copy number of [rib7]. The 4.6 and 8.5 kb EcoRI bands are from the *rosy* locus at 87D. The 8.6 kb HindIII band is from the *white* locus.

(B and C) The X-Y disjunction percentage (B) and the Y chromosome recovery percentage (C) plotted against ectopic rDNA dose. Open circles represent results of spermatocyte squash experiments; closed circles represent progeny count results. Bars indicate 95% confidence intervals. Three independent *Df(1)X-1* lines are represented in the 0 dose bracket. They are the sibling line controls for the single dose insertions at (in order, from left to right) 1A1–4, 1A5–8, and 2EF (see Figure 4).

approximately 50% (random) in the 0 dose lines to approximately 75% in the 2 dose lines. All differences between disjunction percentages from lines at different rDNA dose levels are statistically significant at the .01 level (using the z test); with the exception of the 2 dose lines, values from lines at the same dose level are not significantly different. The 2 dose line with the 2 copies in the same region of the X gave a slightly but significantly higher disjunction percentage in the progeny test than did the line with the more dispersed copies (74% versus 72%). This may indicate that closely linked pairing sites cooperate more effectively than more distantly linked ones.

Progeny ratio distortion exhibited a similar, though more dramatic dependence on rDNA dose. Y chromosome recovery improved from less than 10% in the 0 dose lines to almost 80% in the 2 dose lines; all differences between lines at different dose levels were highly significant (z test) (Figure 6C). Smaller but significant differences were also seen between the 2EF 1 dose insertion and each of the other two single-copy lines. This suggests that position on the X may influence the degree to which a single copy ribosomal gene insertion can rescue progeny ratio distortion. The level of progeny ratio distortion also differed significantly between the 2-copy lines—a fact that is consistent with the proximity effect suggested above.

Discussion

The ability of a single rRNA gene to rescue the pairing and disjunctional defects of a heterochromatically deficient X indicates that the nucleolus organizers function as X-Y pairing sites in normal Drosophila males. Alternative explanations for the pairing effects of [rib7] insertions, such as the presence of closely linked modifiers or pairing activity of P and/or *rosy* sequences, have been ruled out by showing that removal of [rib7] from the X by any of three methods eliminates its ability to pair with the Y and that X insertions of transposons containing only P and *rosy* sequences are powerless to affect X-Y pairing ability. The failure of autosomal [rib7] inserts to stimulate X-Y pairing and the fact that insert-containing X chromosomes pair at the site of insertion together establish that the X rDNA functions as a pairing site rather than as a *trans*-acting contributor to X-Y bivalent formation. The demonstration that the level of X chromosome pairing ability is proportional to ectopic rDNA dose implies that rDNA sequence repetition in the nucleolus organizer is important for full activity in X-Y pairing. The threshold for wild-type pairing is clearly higher than 2 doses since X chromosomes with 2 doses of the rDNA transposon still show substantial levels of nondisjunction. Extrapolating the data in Figure 6B to 100% disjunction would lead to an estimate of 4–6 copies, which is in agreement with the 6–8 copies estimated for one *bobbed-lethal*, pairing-positive deficiency using in situ hybridization to mitotic chromosomes (Appels and Hilliker, 1982). These results provide a plausible explanation for the observation that many *bobbed-lethal* deficiencies retain partial X-Y pairing ability (McKee and Lindsley, 1987). Only those that are completely deficient in rDNA would be expected to lack pairing ability altogether.

In addition to its obvious role in disjunction, X-Y pairing is evidently required for normal sperm development. The fact that the same 12 kb DNA fragment rescues both meiotic defects associated with X heterochromatic deficiencies (X-Y nondisjunction and progeny ratio distortion) strongly implies that they are functionally related. This conclusion is consistent with a large body of evidence (for reviews see McKee and Lindsley, 1987; McKee, 1990) indicative of a causal relationship between X-Y pairing failure and progeny ratio distortion. The nature of this relationship remains a mystery.

In summary, these results provide molecular identification of a meiotic pairing site. They also confirm cytogenetic evidence that the presence of the pairing site is needed both for chromosome disjunction and for normal sperm development.

rDNA in Drosophila Female Meiotic Pairing

Drosophila males are unusual, though not unique, in achieving disjunction without the aid of either chiasmata or synaptonemal complexes. What role might rDNA play in chiasmatic meiotic pairing? In Drosophila females, the role of rDNA in exchange pairing is unclear; deletion of the NO does not interfere with disjunction of normal Xs. However, rDNA may participate in the distributive system that functions to disjoin nonexchange chromosomes. The ability of heterochromatic free X duplications both to disjoin from an attached X and to induce nondisjunction of structurally heterologous free X chromosomes is strongly correlated with the presence of a *bobbed* locus (the NO) on the free duplication (Lindsley and Sandler, 1958). Thus, rDNA may play a major role in achiasmatic pairing in "normal" as well as in obligate achiasmatic meiotic systems.

X-Y Pairing Is Sequence-Specific

Does the nucleolus organizer promote X-Y pairing because of a special property of rRNA genes or simply because it provides homology between the X and Y chromosomes? An approach to this question is to ask whether other sequences common to the X and Y could substitute for the rDNA with respect to pairing. While no other sequences have been tested yet by transformation, the sex chromosomes used in the experiments described above do share homology for sequences other than the rDNA.

Homology for euchromatic sequences is provided by the y^+ and B^S duplications present on B^SYy^+, which are translocations of wild-type X sequences to the tips of YS and YL, respectively. The y^+ duplication includes polytene bands 1A1–1B1 (nine complementation groups [Lindsley and Zimm, 1987]) and contains at least 320 kb, based on pulsed-field Southern analysis of the comparable region of the free duplication, Dp(1; f)1187 (G. H. K. and A. Spradling, unpublished data). The B^S duplication consists of several euchromatic bands from 16A and 20F (Lindsley and Zimm, 1987) of unknown molecular size. *Df(1)X-1* is genetically wild type for all of the duplicated material except part of 20F (Schalet and Lefevre, 1976). The inability of *Df(1)X-1* and B^SYy^+ to pair despite these large (at least several hundred kb) regions of homology indi-

cates that male meiotic pairing is at least partly sequence-specific.

Natural homology between the X and Y chromosomes is provided by at least two classes of repeated sequences in addition to the rDNA. Several families of simple, highly repetitive satellite sequences are represented in the heterochromatin of both sex chromosomes (Peacock et al., 1977; Brutlag, 1980); in addition, there are blocks of the more complex (1.2–2.7 kb), moderately repetitive *Stellate* gene family at 12F in the X euchromatin (80–200 copies) and in the long arm of the Y (>100 copies) (Lovett et al., 1983; Livak, 1984; M. Satter and B. D. M., unpublished data). *Df(1)X-1* has not been tested for its satellite content but does contain a *Stellate* locus (B. D. M., unpublished data). Despite some size and restriction site differences, the X and Y *Stellate* copies are strongly cross-homologous in DNA hybridization experiments. *Df(1)X-1* and $B^{S}Yy^{+}$ share more than 100 kb of *Stellate* homology, yet, as shown above, are unable to pair with each other. Several pieces of evidence indicate that the satellites are also ineffective in meiotic pairing. The disjunction-defective X heterochromatic deficiency, $In(1)sc^{4L}sc^{8R}$, has been shown to retain blocks of the 1.688 g/ml (Hilliker and Appels, 1982) and 1.672 g/ml (Steffenson et al., 1981) satellite sequences near the centromere, the latter of which is common to the Y. There are also several free duplications of the X and second chromosomes that lack significant pairing ability and consist mostly of satellite-rich heterochromatin (Lindsley and Sandler, 1958; Yamamoto, 1979). In addition, iso–second chromosomes that overlap only in the heterochromatin disjoin at random from each other (Hilliker and Appels, 1982). The failure of satellite sequences to contribute to chromosome disjunction could be due to their distribution. Except for the 1.688 g/ml family that is X-specific (Hilliker and Appels, 1982), each of the major satellite families is represented in the heterochromatic regions of all of the major chromosomes (Appels and Peacock, 1978). *Stellate*, however, is restricted to the X and Y. Its lack of pairing ability implies that neither tandem repetition nor presence on both the X and Y chromosomes suffices to ensure participation in male meiotic pairing.

How Does the rDNA Promote X-Y Pairing?

The fact that a 12 kb rDNA insert can stimulate X-Y pairing, while other, much larger regions of homology cannot, implies that there is something special about the rDNA with respect to meiotic pairing. The basis for this specificity is unknown. A sequence within the rDNA could act as a binding site for a protein that can interact with similar protein–DNA complexes on the homolog. Alternatively, a region of unique secondary structure could permit direct interactions between homologous rDNA sequences. There are two known features of rRNA genes that could be relevant to pairing: germline transcriptional activity and ability to form a nucleolus.

Transcriptional activity has been associated with a supercoiled and relatively accessible chromatin conformation (Liu and Wang, 1987) and with binding of one or more activating proteins, either of which could predispose a sequence for participation in pairing. Of possible relevance is the mounting evidence that recombination in yeast is stimulated locally by transcription (Voelkel-Meiman et al., 1987; Thomas and Rothstein, 1989). The difference in pairing ability between the rDNA and the *Stellate* complex could be related to transcriptional differences; *Stellate* is expressed at very low levels in XY testes (Livak, 1984).

The nucleolus functions as the transcription site only for rRNA genes, thus effectively isolating them from other chromosomal sequences. Assuming that both the X and Y rRNA genes are present simultaneously in the single nucleolus of the premeiotic primary spermatocyte, this structure could minimize the spatial and informational difficulties involved in homologous recognition. The nucleolus disintegrates during meiosis, but nucleolar components could be modified and used in the X-Y pairing structure. There have been several reports of unusual behavior of nucleoli suggestive of a role in meiotic chromosome pairing and disjunction in other organisms (for examples see Friedlander et al., 1976; Oud and Reutlinger, 1981; Morag et al., 1982). Since the autosomes in Drosophila males pair and disjoin achiasmatically without having nucleolus organizers, it is unlikely that the nucleolus per se could provide a general pairing mechanism. However, it is possible that other stable nuclear structures, such as the snRNP-containing "sphere" organelles recently described in oocyte nuclei of frogs, newts, spiders, and crickets, could play analogous roles in autosomal pairing. In amphibians, some spheres are intimately associated with the chromatin at particular loci, termed the "sphere organizers"; in some cases homologous sphere organizers are associated with the same sphere (Gall and Callan, 1989). The importance of transcription and nucleolus formation in rDNA pairing can be assessed using the single gene system described in this report, combined with deletion mutagenesis. Both transcription and nucleolus formation are autonomous properties of single rRNA genes (Karpen et al., 1988). Molecular deletion mapping of ectopic rRNA sequences responsible for pairing, transcription, and nucleolus formation should reveal whether or not there are functional associations among them. In addition, identification of the rDNA pairing sequence(s) will provide the material for further biochemical and genetic studies, such as the identification of putative pairing proteins or genes whose products interact with the pairing site.

Experimental Procedures

Chromosomes

Df(1)X-1 is a large heterochromatic deficiency with breakpoints proximal to the NO and in the proximal euchromatin distal to or in *l(1)20Cb* in 20F (Lindsley and Zimm, 1986, 1987). Its sequence is otherwise normal. $B^{S}Yy^{+}$ is duplicated for 1A1–1B1 from the tip of the X and for three additional bands from the proximal X (Lindsley and Zimm, 1987). Its meiotic behavior is indistinguishable from that of an unmarked Y. $B^{S}Y$ is constructed similarly but lacks the 1A1–1B1 duplication. SM1, *Cy* and TM3, *Sb* are standard balancers for the second and third chromosomes, respectively. Markers are described in Lindsley and Grell (1968) and Lindsley and Zimm (1985).

Plasmids

p(rib,ry)7 was constructed as described previously (Karpen et al., 1988)

by inserting a 13.2 kb NruI–BalI fragment from clone pKB326 (I. Dawid, personal communication) containing a full-length rDNA repeat into the HpaI site of Carnegie 20 (Car-20) (Rubin and Spradling, 1983). ry11 is identical to Car-20 except for a single restriction site difference in the polycloning region (Spradling and Rubin, 1983). The two ry11 insertion lines were kindly supplied by A. Spradling.

Manipulation of Transposons

The procedures for germline transformation of p(rib,ry)7 and for remobilization of [rib7] insertions were described previously (Karpen et al., 1988). [rib7](1A1–4) was destabilized by exposure for one generation to the genomically integrated transposase source, Δ2,3(99B) (Robertson et al., 1988). The crossing scheme was as follows:

$$P\ \frac{[rib7](1A1\text{–}4),\ ry^{+}}{[rib7](1A1\text{–}4),\ ry^{+}};\ \frac{TM3,\ ry^{RK}\ Sb}{ry^{506}} \times \frac{+}{Y};\ \frac{\Delta 2,3(99B),\ ry^{+}}{\Delta 2,3(99B),\ ry^{+}}$$

$$F1\ \frac{[rib7](1A),\ ry^{+}}{Y};\ \frac{\Delta 2,3(99B),\ ry^{+}}{TM3,\ ry^{RK}\ Sb} \times \frac{FM6,\ y^{2}\ B}{y\ sn\ cor\text{-}36};\ \frac{ry^{506}}{ry^{506}}$$

$$F2\ \frac{ry^{-}}{FM6,\ y^{2}\ B};\ \frac{TM3,ry^{RK}Sb}{ry^{506}}.$$

In this experiment, only *rosy*⁻ F2 females were selected; many of these (16 out of 27 *ry*⁻ females) proved to be completely deficient for [rib7] and were used for the reversion tests. In another experiment, *rosy*⁺ F2 females were also recovered. While many of these contained an unaltered [rib7](1A1–4), four lines (out of 41 *ry*⁺ lines) were found to contain second-site insertions of [rib7] that were used in the comparison of X-linked and autosomal insertions and in the study of dose effects.

Genomic Southern Blot Analysis

DNA for genomic blots was prepared from 4–6 flies by the method of Bender et al. (1983). Genomic DNA was digested 2–4 hr with the appropriate restriction enzyme (purchased from Bethesda Research Laboratories), electrophoresed on 0.5% agarose gels in Tris–Borate–EDTA buffer (Maniatis et al., 1982), and transferred to Gene Screen Plus filters by the Southern blot method (Southern, 1975) according to the manufacturer's (New England Nuclear) instructions. After prehybridization for 15 min in 50% formamide, 1% SDS, and 5× SSPE (Maniatis et al., 1982), denatured probe DNA, prepared by nick translation (using a kit from Bethesda Research Laboratories) in the presence of [^{32}P]dCTP (from New England Nuclear), was added. Hybridization was carried out overnight at 42°C. Filters were washed twice at room temperature in 2× SSC, twice at 65°C in 2× SSC, 1% SDS, and twice at room temperature in 0.1× SSC, and exposed at −70°C to Kodak XAR-5 film for varying amounts of time.

In Situ Hybridization

Larvae for polytene chromosome analysis were cultured in standard cornmeal-molasses-yeast agar at 18°C under uncrowded conditions. Salivary gland squashes and in situ hybridization were carried out according to the method of Johnson-Schlitz and Lim (1987). Probe DNA was labeled by nick translation in the presence of [^{3}H]dTTP (Amersham).

Meiotic Chromosome Preparations

Male meiotic chromosomes were prepared by the method of Lifschytz and Hareven (1977). In brief, testes from 0–2 day old adults were dissected in Shen's solution, fixed for 30 s in 45% acetic acid, stained for 5 min in 3% orcein–60% acetic acid, transferred to a drop of 60% acetic acid on a microscope slide, sliced with a sharp dissecting needle near the apical end, and gently squashed with a cover slip containing a drop of 2% lactic–acetic–orcein. Well-stained and spread figures were examined under phase-contrast optics using a Zeiss Universal Axioplan photomicroscope and photographed using Kodak T-MAX 100 film.

Progeny Tests

Males were crossed singly to two *rosy*⁻ females in vials containing cornmeal-molasses-yeast agar and cultured at 22°C–23°C. The parents were transferred to fresh medium on day 6 or 7 and discarded on day 12 or 13. Progeny in the first vial were counted on days 12, 15, and 19 and in the second vial on days 19, 22, and 25.

Parameters and Statistics

The X-Y disjunction frequency (P) is defined as the fraction of secondary spermatocytes that receive an X or a Y but not both ((X + Y)/(X + Y + XY + O)). It was estimated from testis squash data using the formula P = (2a + d)/(2a + 2b + c + d + e) (see Table 1 for definitions). The variance is V = P(1−P)/N.

The disjunction frequency was estimated from progeny class frequencies by the method of McKee (1984). This method corrects for skewed progeny class frequencies caused by sperm abortion by estimating chromosome viability parameters R_X and R_Y, defined as the viability of sperm that carry the relevant chromosome divided by the viability of otherwise identical sperm that lack it. Using these two parameters in addition to the disjunction frequency P, the expected frequencies of the four sperm classes (X, Y, XY, and O) in the progeny can be written as: (1) e(X) = PR_X/S; (2) e(Y) = PR_Y/S; (3) e(XY) = $(1-P)R_XR_Y/S$; and (4) e(O) = $(1-P)/S$ where $S = PR_X + PR_Y + (1-P)R_XR_Y + (1-P)$. This model treats the chromosome viability parameters as independent of each other and of the disjunction frequency. The validity of this assumption has been established experimentally (McKee, 1984; McKee and Lindsley, 1987). Estimates of P, R_X, and R_Y can be obtained by replacing e(i) in equations 1–4 with the observed class frequencies o(i) and solving the equations simultaneously. The solutions are:

$$P = 1/[1 + (o(XY)o(O)/o(X)o(Y))^{1/2}];$$
$$R_X = [(o(XY)o(X))/(o(Y)o(O))]^{1/2}; \text{ and}$$
$$R_Y = [(o(XY)o(Y))/(o(X)o(O))]^{1/2}.$$

These are maximum likelihood estimates. The variances are:

$$V_j = 1/[\Sigma_i((1/e(i))(\partial e(i)/\partial j)^2)N],$$

where i represents the progeny classes and j represents the parameters (Kempthorne, 1969).

For both assays, pairwise comparisons of parameters obtained from crosses involving different lines were made using the statistic:

$$z = (j_1 - j_2)/(V(j_1) + V(j_2))^{1/2}.$$

Acknowledgments

We gratefully acknowledge Julie Vrana, Cynthia Merrill, and Kathy Wilhelm for very capable technical assistance, Dr. Johng K. Lim for advice on cytogenetic procedures, Dr. Yui Tim Ho for advice on statistics, and Carol Massoth for assistance with graphics. We also thank Dr. Charles Laird for his encouragement during the early stages of this work, and Dr. Dennis McKearin, Dr. Dan Lindsley, and two reviewers for critical readings of the manuscript. This work was supported by grants to B. D. M. from the National Institute of Child Health and Human Development (#1R15HD2122501) and the National Institute of General Medical Sciences (#1R01GM4048901). B. D. M. is supported by a Research Career Development Award (5K04GM0057202) from the National Institute of General Medical Sciences. G. H. K. is supported by National Institutes of Health postdoctoral fellowship GM1180203.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *"advertisement"* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received November 30, 1989; revised January 25, 1990.

References

Appels, R., and Hilliker, A. J. (1982). The cytogenetic boundaries of the rDNA region within heterochromatin of the *X* chromosome of *Drosophila melanogaster* and their relation to the male meiotic pairing sites. Genet. Res. *39*, 149–156.

Appels, R., and Peacock, W. J. (1978). The arrangement and evolution of highly repeated (satellite) DNA sequences with special reference to *Drosophila*. Int. Rev. Cytol. Suppl. *8*, 69–126.

Ault, J. G., Lin, H. P., and Church, K. (1982). Meiosis in *Drosophila melanogaster*. IV. Conjunctive mechanism of the *X-Y* bivalent. Chromosoma *86*, 309–317.

Baker, B. S., and Carpenter, A. T. C. (1972). Genetic analysis of sex chromosomal meiotic mutants in *Drosophila melanogaster*. Genetics *71*, 255–286.

Bender, W., Spierer, P., and Hogness, D. S. (1983). Chromosome walking and jumping to isolate DNA from the *Ace* and *rosy* loci and the *bithorax* complex in *Drosophila melanogaster*. J. Mol. Biol. *168*, 17–33.

Brutlag, D. L. (1980). Molecular arrangement and evolution of heterochromatic DNA. Annu. Rev. Genet. *14*, 121–144.

Cooper, K. W. (1964). Meiotic conjunctive elements not involving chiasmata. Proc. Natl. Acad. Sci. USA *52*, 1248–1255.

Craymer, L. (1981). Techniques for manipulating chromosome rearrangements and their application to *Drosophila melanogaster*. I. Pericentric inversions. Genetics *99*, 75–97.

Friedlander, M., Gershon, J., and Reinhartz, A. (1976). Atypical cycle of the nucleolus in spermatocytes of the insect *Locusta migratoria*. Cytobiologie *13*, 171–181.

Gall, J. G., and Callan, H. G. (1989). The sphere organelle contains small nuclear ribonucleoproteins. Proc. Natl. Acad. Sci. USA *86*, 6635–6639.

Gershenson, S. (1933). Studies on the genetically inert region of the *X* chromosome of *Drosophila*. I. Behavior of an *X* chromosome deficient for a part of the inert region. J. Genet. *28*, 297–312.

Giroux, C. N. (1988). Chromosome synapsis and meiotic recombination. In Genetic Recombination, R. Kucherlapati and G. R. Smith, eds. (Washington: American Society for Microbiology), pp. 465–496.

Grell, R. F. (1976). Distributive pairing. In The Genetics and Biology of *Drosophila*, Vol. 1A, M. Ashburner and E. Novitski, eds. (London: Academic Press), pp. 435–486.

Hawley, R. S. (1988). Exchange and chromosomal segregation in eucaryotes. In Genetic Recombination, R. Kucherlapati and G. R. Smith, eds. (Washington: American Society for Microbiology), pp. 497–527.

Hilliker, A. J., and Appels, R. (1982). Pleiotropic effects associated with the deletion of heterochromatin surrounding rDNA on the *X* chromosome of *Drosophila*. Chromosoma *86*, 469–480.

Hilliker, A. J., Holm, D. G., and Appels, R. (1982). The relationship between heterochromatic homology and meiotic segregation of compound second autosomes during spermatogenesis in *Drosophila melanogaster*. Genet. Res. *39*, 157–168.

Holm, D. G. (1976). Compound autosomes. In The Genetics and Biology of *Drosophila*, Vol. 1B, M. Ashburner and E. Novitski, eds. (London: Academic Press), pp. 529–561.

Johnson-Schlitz, D., and Lim, J. K. (1987). Cytogenetics of *Notch* mutations arising in the unstable *X* chromosome *Uc* of *Drosophila melanogaster*. Genetics *115*, 701–709.

Karpen, G. H. (1987). The relationship between organization and function of ribosomal genes in *Drosophila melanogaster*. Ph.D. thesis, University of Washington, Seattle, Washington.

Karpen, G. H., Schaeffer, J. E., and Laird, C. D. (1988). A *Drosophila* rRNA gene located in euchromatin is active in transcription and nucleolus formation. Genes Dev. *2*, 1745–1763.

Kempthorne, O. (1969). An Introduction to Genetic Statistics (Ames, Iowa: The Iowa State University Press).

Lifschytz, E., and Hareven, A. (1977). Gene expression and the control of spermatid morphogenesis in *Drosophila melanogaster*. Dev. Biol. *58*, 276–294.

Lindsley, D. L., and Grell, E. H. (1968). Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 627.

Lindsley, D. L., and Sandler, L. (1958). The meiotic behavior of grossly deleted *X* chromosomes in *Drosophila melanogaster*. Genetics *43*, 547–563.

Lindsley, D. L., and Zimm, G. (1985). The genome of *Drosophila melanogaster*, Part 1. Dros. Inf. Serv. *62*.

Lindsley, D. L., and Zimm, G. (1986). The genome of *Drosophila melanogaster*, Part 2. Dros. Inf. Serv. *64*.

Lindsley, D. L., and Zimm, G. (1987). The genome of *Drosophila melanogaster*, Part 3. Dros. Inf. Serv. *65*.

Lindsley, D. L., Appels, R., and Hilliker, A. J. (1982). *In(1)sc^{V2}* subdivides the ribosomal DNA. Dros. Inf. Serv. *58*, 99.

Liu, L. F., and Wang, J. C. (1987). Supercoiling of the DNA template during transcription. Proc. Natl. Acad. Sci. USA *84*, 7024–7027.

Livak, K. J. (1984). Organization and mapping of a sequence on the *Drosophila melanogaster X* and *Y* chromosomes that is transcribed during spermatogenesis. Genetics *107*, 611–634.

Long, E. O., and Dawid, I. B. (1980). Repeated genes in eucaryotes. Annu. Rev. Biochem. *49*, 727–764.

Lovett, J. A., Kaufmann, T. C., and Mahowald, A. P. (1983). A locus on the *X* chromosome apparently controlled by the *Y* chromosome during spermatogenesis in *Drosophila melanogaster*. Eur. J. Cell Biol. *22*, 49.

Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982). Molecular Cloning: A Laboratory Manual (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

McIntosh, J. R. (1983). The centrosome as an organizer of the cytoskeleton. Mod. Cell Biol. *2*, 115–142.

McKee, B. D. (1984). Sex chromosome meiotic drive in *Drosophila melanogaster* males. Genetics *106*, 403–422.

McKee, B. D. (1990). *X-Y* pairing, meiotic drive, and ribosomal DNA in *Drosophila melanogaster* males. Am. Natural., in press.

McKee, B., and Lindsley, D. L. (1987). Inseparability of *X*-heterochromatic functions responsible for *X:Y* pairing, meiotic drive and male fertility in *Drosophila melanogaster*. Genetics *116*, 399–407.

Meyer, G. F. (1960). The fine structure of spermatocyte nuclei of *Drosophila melanogaster*. In Proceedings of the European Regional Conference on Electron Microscopy, A. L. Houwink and B. J. Spit, eds. (Delft: Die Nederlandse Vereniging voor Electronmicroscopic Delft), pp. 951–954.

Mitchison, T. (1988). Microtubule dynamics and kinetochore function. Annu. Rev. Cell Biol. *4*, 527–549.

Morag, D., Friedlander, M., and Raveh, D. (1982). Synaptonemal complexes, telomeric nucleoli, and the karyosphere in achiasmatic oocyte meiosis of the carob moth. Chromosoma *87*, 293–302.

Morgan, T. H. (1912). Complete linkage in the second chromosome of the male of *Drosophila melanogaster*. Science *36*, 719–720.

Muller, H. J., and Painter, T. S. (1932). The differentiation of the sex chromosomes of *Drosophila* into genetically active and inert regions. Z. Indukt. Abstammungs Vererbungsl. *62*, 316–365.

Oud, J. L., and Reutlinger, A. H. H. (1981). The behavior of silver positive structures during meiotic prophase of male mice. Chromosoma *81*, 569–578.

Peacock, W. J. (1965). Nonrandom segregation of chromosomes in *Drosophila* males. Genetics *51*, 573–583.

Peacock, W. J., and Miklos, G. L. G. (1973). Meiotic drive in *Drosophila*: new interpretations of the *Segregation Distorter* and sex chromosome systems. Adv. Genet. *17*, 361–409.

Peacock, W. J., Miklos, G. L. G., and Goodchild, D. J. (1975). Sex chromosome meiotic drive systems in *Drosophila melanogaster*. I. Abnormal spermatid development in males with a heterochromatin deficient *X* chromosome (*sc^{4}sc^8*). Genetics *79*, 613–634.

Peacock, W. J., Lohe, A. R., Gerlach, W. L., Dunsmuir, P., Dennis, E. S., and Appels, R. (1977). Fine structure and evolution of DNA in heterochromatin. Cold Spring Harbor Symp. Quant. Biol. *42*, 1121–1135.

Rieder, C. L. (1982). The formation, structure and composition of the mammalian kinetochore and kinetochore fiber. Int. Rev. Cytol. *79*, 1–58.

Ritossa, F. (1976). The *bobbed* locus. In The Genetics and Biology of *Drosophila*, Vol. 1B, M. Ashburner and E. Novitski, eds. (London: Academic Press), pp. 801–846.

Robertson, H. M., Preston, C. R., Phillis, R. W., Johnson-Schlitz, D. M., Benz, W. K., and Engels, W. R. (1988). A stable genomic source of P element transposase in *Drosophila melanogaster*. Genetics *118*, 461–470.

Rubin, G. M., and Spradling, A. C. (1983). Vectors for P-element mediated gene transfer in *Drosophila*. Nucl. Acids Res. *11*, 6341–6351.

Sandler, L., and Braver, G. (1954). The meiotic loss of unpaired chromosomes in *Drosophila melanogaster*. Genetics *39*, 365–377.

Schalet, A., and Lefevre, G. (1976). The proximal region of the X chromosome. In The Genetics and Biology of *Drosophila*, Vol. 1B, M. Ashburner and E. Novitski, eds. (New York: Academic Press), pp. 847–902.

Southern, E. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. *98*, 503–517.

Spradling, A. C., and Rubin, G. M. (1983). The effect of chromosomal position on the expression of the Drosophila xanthine dehydrogenase gene. Cell *34*, 47–57.

Steffenson, D. M., Appels, R., and Peacock, W. J. (1981). The chromosomal distribution of the 1.705 g/cc and 1.672 g/cc satellite DNA's in *Drosophila melanogaster*. Chromosoma *82*, 525–541.

Thomas, B. J., and Rothstein, R. (1989). Elevated recombination rates in transcriptionally active DNA. Cell *56*, 619–630.

Voelkel-Meiman, K., Keil, R. L., and Roeder, G. S. (1987). Recombination-stimulating sequences in yeast ribosomal DNA correspond to sequences regulating transcription by RNA polymerase I. Cell *48*, 1071–1079.

von Wettstein, D., Rasmussen, S. W., and Holm, P. B. (1984). The synaptonemal complex in genetic segregation. Annu. Rev. Genet. *18*, 331–414.

Yamamoto, M. (1979). Cytological studies of heterochromatin function in *Drosophila melanogaster* male: autosomal meiotic pairing. Chromosoma *72*, 293–328.

Zimmering, S. (1963). The effect of temperature on meiotic loss of the *Y* chromosome in the male *Drosophila*. Genetics *48*, 133–138.